Regulators and effectors of the ARF GTPases

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The small G proteins of the ARF family are key regulators of membrane dynamics. Many functions of ARF proteins in cells are being revealed by studies of their regulators and effectors. Significant progress has been made over the past year, with the identification of a surprisingly large family of novel ARF GTPase-activating proteins. In addition, two new classes of effectors, the PIP kinases and a novel family of monomeric coat-like proteins have been discovered.

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Abbreviations

ARF	ADP-ribosylation factor
ARNO	ARF nucleotide-binding site opener
ASAP1	ARF GAP containing SH3, ANK repeat and PH domains 1
BFA	brefeldin A
BIG	brefeldin A-inhibited GEF
COPI	coat protein complex I
GAP	GTPase-activating protein
GBF1	Golgi BFA resistance factor 1
GEF	guanine nucleotide exchange factor
GGA	Golgi-localizing, gamma-adaptin ear homology domain ARF-binding protein
GRK	G-protein-coupled receptor kinase
GRP1	general receptor for phosphoinositides 1
MDCK	Madin-Darby canine kidney
mw	molecular weight
myrARF1	myristoylated ARF1
PH	pleckstrin homology
PI	phosphoinositide
PIP	phosphatidylinositol phosphate
PIP_2	phosphatidylinositol 4,5-bisphosphate
PIP_3	phosphatidylinositol 3,4,5-triphosphate
PLD	phospholipase D
PM	plasma membrane
TGN	*trans*-Golgi network
VHS	Vps27, Hrs and STAM domain

Introduction

ADP-ribosylation factors (ARFs) are small (approximately 20 kDa) guanine-nucleotide-binding proteins that regulate membrane traffic and organelle structure in eukaryotic cells. In general, the inactive GDP-bound form of ARF is soluble, although it can associate weakly with membranes, whereas the active GTP-bound form binds tightly to the membrane. ARFs function on membrane surfaces where they encounter their effectors and regulators, the guanine nucleotide exchange factors (GEFs) and GTPase-activating proteins (GAPs). ARF effectors include lipid-modifying enzymes and cytosolic coat complexes (such as COPI) that are recruited onto membranes by ARF-GTP. Hence, ARF activation leads to changes in both the lipid and protein composition of the membrane on which it is localized; changes which in turn result in modulation of membrane structure and function.

ARF proteins are highly conserved and have been found in all eukaryotic organisms examined. Mammalian ARF proteins are divided into three classes: Class I (ARF1–ARF3), Class II (ARF4 and ARF5) and Class III (ARF6). In the yeast *Saccharomyces cerevisiae*, there are three ARF proteins. Arf1 and Arf2 are functionally interchangeable, and yeast cells require at least one of these proteins for viability. Yeast Arf3 is not essential for growth and probably corresponds to mammalian ARF6. Both *Drosophila melanogaster* and *Caenorhabditis elegans* have at least one orthologue of each of the three classes of mammalian ARFs.

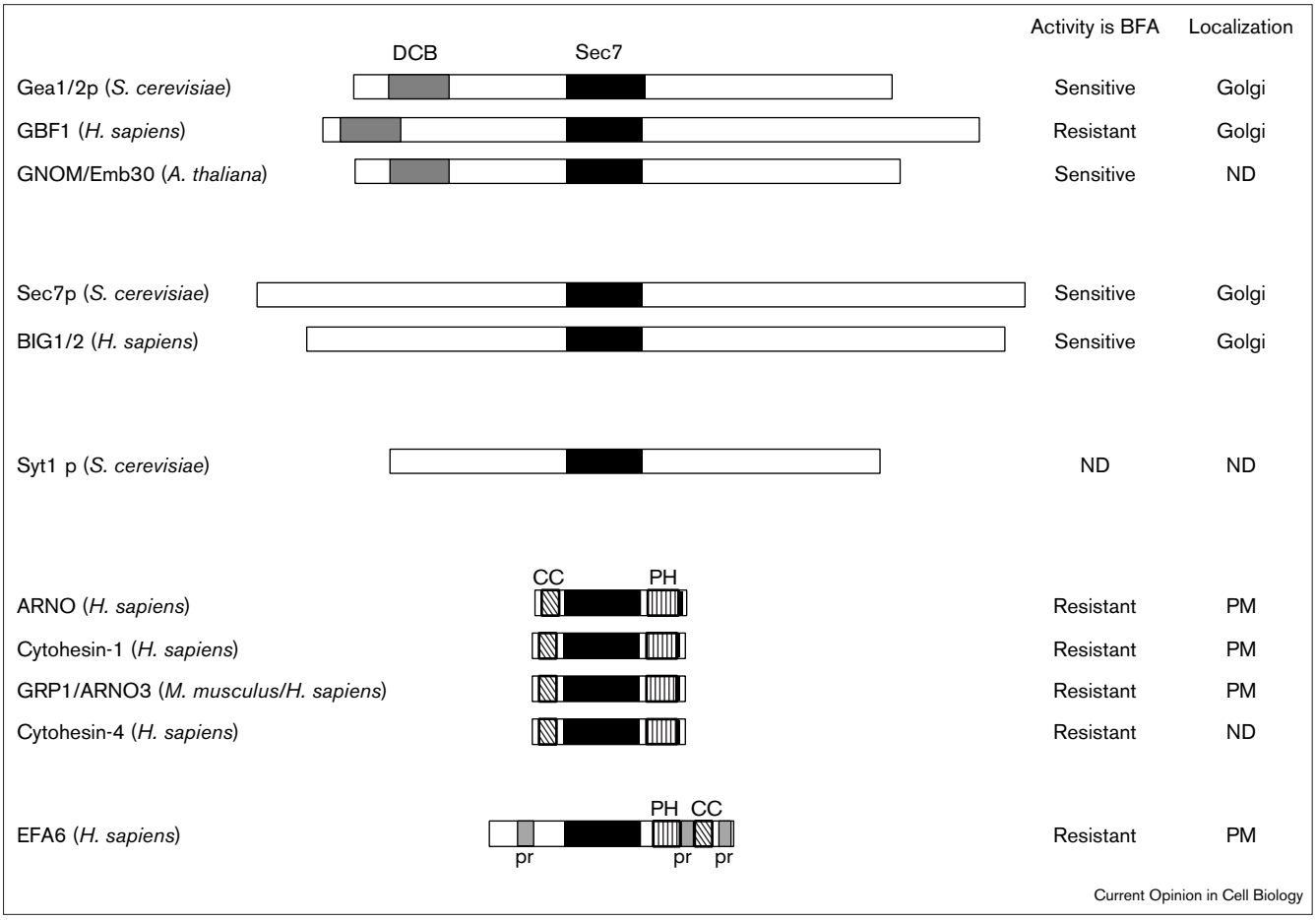
Class I ARFs are involved in trafficking in the ER–Golgi and endosomal systems, and their functions have been extensively studied (for reviews see [1–4]). ARF1 binding to endosomal membranes is regulated by endosomal pH, which explains the pH dependence of COPI binding to endosomes [5•]. The Class III ARF, ARF6, functions exclusively in the endosomal–plasma membrane system. ARF6 is involved in endosomal recycling to the plasma membrane (PM), in regulated secretion, and in coordinating actin cytoskeleton changes at the PM (see [1]). ARF6 is present at the apical surface of MDCK cells, where it plays a role in modulating clathrin endocytosis ([6•]; Mostov *et al.*, pp 483–490). ARF6 has also been implicated in Fc-mediated phagocytosis in macrophages [7] and in insulin stimulation of adipsin secretion [8] and Glut4 translocation [9]. By contrast, virtually nothing is known about the functions of the class II ARFs.

Guanine nucleotide exchange factors

All ARF GEFs identified to date possess a Sec7 domain, a module of approximately 200 amino acids that is sufficient to catalyze exchange of GDP for GTP on ARF *in vitro* (Figure 1). The Sec7 family of proteins has been reviewed recently [10,11], so we will briefly highlight studies published over the last year. The high mw ARF GEFs of the Gea/GBF/GNOM and Sec7/BIG subfamilies function in the ER–Golgi system, whereas the ARNO/cytohesin/GRP and EFA6 subfamilies function primarily in the endosomal–PM system. The yeast GEF Syt1 represents a novel subfamily [12•]. The fungal metabolite brefeldin A (BFA), known to disassemble the Golgi complex and block secretion, directly inhibits some of the ARF GEFs, including the majority of the large ARF GEFs, but has little effect on the activity of the low mw GEFs (see Figure 1). The target of BFA is an ARF–GDP–GEF reaction intermediate that BFA stabilizes, thus blocking the cycle of activation of ARF [13,14].

Membranes play an essential role in ARF activation. ARF must first undergo a lipid-mediated conformational

Figure 1



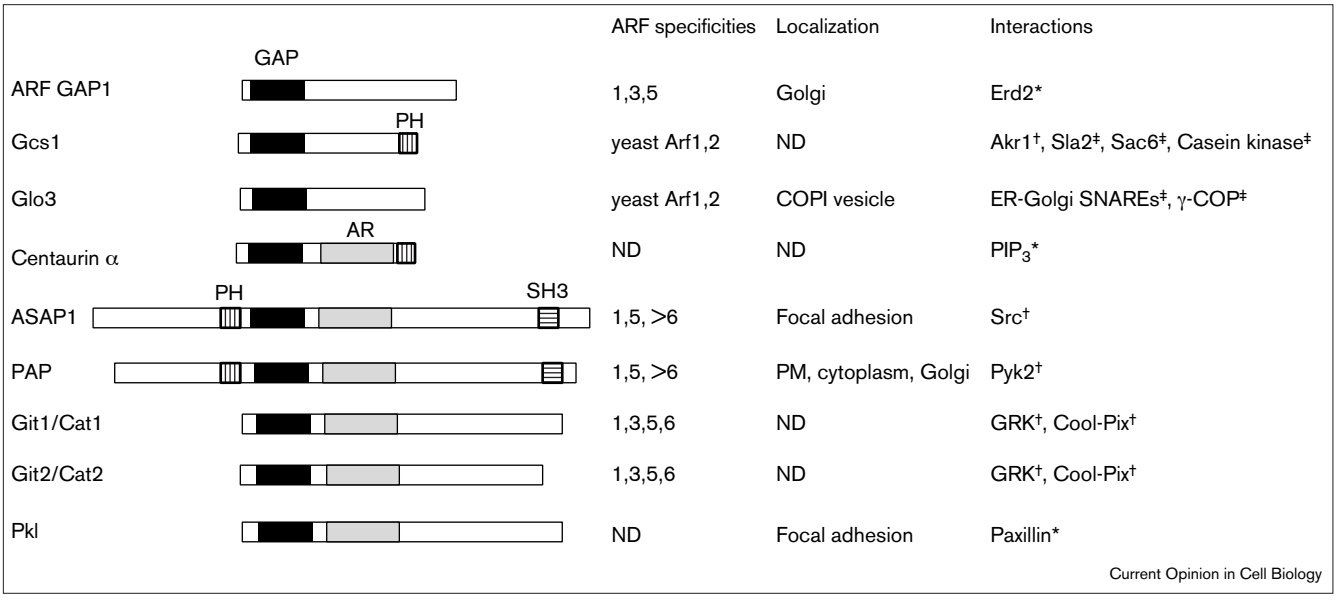
The Sec7 family of ARF GEFs. The black box represents the Sec7 domain, which catalyzes GTP/GDP exchange on ARF. The DCB domain (for dimerization and cyclophilin binding, grey) mediates interactions between GNOM monomers and also directly binds to the cyclophilin Cyp5 [67]. The PH domain (PH, vertical stripes) and coiled-coil regions (CC, diagonal stripes) of the lower mw GEFs are indicated. The solid bar following some PH domains designates the polybasic region. EFA6 contains proline-rich regions designated 'pr'. Brefeldin A (BFA) inhibits the *in vitro* ARF exchange activity of certain GEFs (indicated 'Sensitive') but not of others (indicated 'Resistant'). The major cellular localization of each GEF as determined by immunofluorescence is indicated. ND, not determined.

switch before it can form a productive complex with a membrane-associated GEF [15•]. Hence, ARF activation takes place after both the GEF and its target ARF have been localized to membranes. All ARF GEFs identified to date are soluble proteins that are peripherally associated with membranes. BIG1, BIG2 and GBF1, high mw GEFs, are all localized to the Golgi apparatus in mammalian cells [14,16•,17•]. An amino-terminal fragment of human BIG1 localizes to the Golgi, indicating that this portion of the protein has membrane-targeting information [14]. Yeast Sec7 localization to membranes is important for transport in the ER–Golgi system [18]. The GNOM/Emb30 ARF GEF of *Arabidopsis thaliana* is necessary for polarized PM localization of the auxin efflux carrier PIN1 [19•].

The low mw subfamily of ARF GEFs are involved in signalling pathways downstream of PI 3-kinases [20, 21], in actin cytoskeleton remodelling [22] and in integrin signalling [23•]. All ARNO/cytohesin/GRP family members contain a pleckstrin homology (PH) domain that mediates membrane localization via interaction with specific polyphosphoinositides and an adjacent carboxy-terminal polybasic domain that cooperates with the PH domain to enhance membrane binding. Membrane binding of ARNO is negatively regulated by PKC-mediated phosphorylation of a serine residue within this polybasic domain [24•].

A key question that is still difficult to answer *in vivo* is the specificity of the different GEFs for the different ARFs. In several *in vitro* systems, ARNO, cytohesin-1 and GRP1 catalyze exchange more efficiently on class I ARFs than members of the other classes (see [10]). However, *in vivo*, ARNO and GRP1 colocalize with ARF6 and play an important role in ARF6 activation [21,22,25•]. A new member of this family, cytohesin-4, efficiently catalyzes exchange on ARF1, and to a lesser extent on ARF5 *in vitro*, but it is inactive towards ARF6 [26]. The situation is much clearer for

Figure 2



Schematic representation of the ARF GAP family. Gcs1 and Glo3 are from *S. cerevisiae*; the others are all mammalian proteins. All proteins share homology in the GAP domain (black) that contains a critical zinc finger motif. A number of the GAPs shown also have a PH domain (vertical stripes); these GAPs have been referred to as centaurins. There are many more centaurin sequences in the databases that have yet to be characterized. Ankyrin repeats (AR, grey) and src homology 3 (SH3) domains (horizontal stripes) are indicated. The ARF substrate specificities reported are from *in vitro* assays. Centaurin α was identified as a PIP_3-binding protein; ASAP as an interacting partner with SH3 domains of Src; and PAP as an interacting partner with the tyrosine kinase Pyk2. Git1 and Git2 are identical to Cat1 and Cat2, identified independently by interactions with GRK and Cool-Pix, respectively. The method used to establish the indicated interactions is as follows: *biochemical; †two-hybrid; ‡yeast genetic. ND, not determined.

EFA6, which activates ARF6 more efficiently than ARF1 *in vitro* and is involved (like ARF6) in endosome–PM recycling and actin cytoskeleton remodelling [27].

GTPase-activating proteins

GAPs stimulate ARF-bound GTP hydrolysis and, hence, return ARF to the inactive GDP-bound state. The timing of GAP activity is critical for the function of GTPases, and in some cases, GAPs can participate in effector functions. During the past year, many new ARF GAP proteins have been identified (see [28]). These new ARF GAPs are multi-domain proteins that were identified, in many cases, as binding partners of signal transduction molecules (Figure 2). All of these proteins share a common GAP domain of 70 amino acids, which includes a zinc finger motif of CXXCX(16-17)CXXC (where C is cysteine and X is any amino acid) that is critical for GAP activity [29].

In addition to the zinc finger, all ARF GAPs have a conserved arginine within the GAP domain. Mutation of this arginine to lysine results in a 100,000-fold decrease in GTPase activity for ASAP1 [30•] and for its close relative, PAPβ [31•], indicating that this arginine is essential for GAP activity and suggesting an arginine finger mechanism for GTP hydrolysis. A recent crystal structure of the GAP and ankyrin repeat domains of PAPβ reveals that this arginine is positioned on the surface of the molecule near several hydrophobic residues [31•]. Mutation of these adjacent hydrophobic residues also impairs GAP activity, suggesting that this region may represent the ARF interaction site [31•]. However, a crystal structure of ARF GAP1 complexed to ARF1-GDP indicates a different site of interaction between ARF and GAP [32], distant from this critical arginine. Further studies will be needed to resolve the discrepancy between the two proposed ARF interaction sites and mechanisms of catalysis.

Goldberg ([32]; see also update) provides evidence that the effector COPI complex may participate in the GAP reaction of ARF GAP1. This is in line with a general model proposed by Schekman and colleagues whereby coat complexes include GAP activity that would be regulated by cargo molecules after coat recruitment to membranes [33]. Evidence now exists for cargo regulation of ARF hydrolysis through COPI at the Golgi [34•] and by the mannose 6-phosphate receptor at the TGN [35].

ARF GAP1, the first GAP for ARF to be cloned, is localized to the Golgi complex in mammalian cells where it acts on ARF1 [29]. Moderate overexpression of ARF GAP1 in cells results in increased GTP hydrolysis and shorter residency time of ARF1 on the Golgi complex [36•]. Higher levels of overexpression result in phenotypes characteristic of the loss of active ARF1 at the Golgi, as induced by either BFA

treatment or expression of the GTP-binding-defective $ARF1^{T31N}$ mutant [37]. ARF GAP1 can be recruited to Golgi membranes by the KDEL receptor Erd2, suggesting that GAP recruitment may be involved in retrograde transport back to the ER stimulated by KDEL proteins [36•,37].

In yeast, the two ARF GAPs Gcs1 and Glo3 have been implicated in retrograde, Golgi-to-ER, transport [38•]. Glo3 was identified independently as a Golgi-to-ER retrieval (*ret*) mutant in yeast, indicating a role in retrieval of KKXX-bearing ER resident proteins after they escape into the Golgi [39]. Moreover, the importance of GAP-mediated GTP hydrolysis on ARF is underscored by recent observations that vesicles generated *in vitro* in the presence of GTP contain a higher concentration of cargo proteins than those prepared with GTPγS or the constitutively active ARF^{Q71L} [40•,41•].

A striking parallel to the observation that ARF-GAP1 is recruited to the Golgi by Erd2, a seven-pass transmembrane receptor, is that a peripheral ARF GAP, Git1, is recruited to G-protein-coupled receptors (also a seven-pass transmembrane proteins) at the PM. Git1 was isolated as a binding partner of G-protein-coupled receptor kinases (GRKs) [42], which regulate receptor internalization following ligand stimulation. Git1 is a PIP_3-stimulated GAP for all ARFs including ARF6 [43•]. Git1 overexpression specifically inhibits internalization of G-protein-coupled receptors that are normally internalized via clathrin-facilitated endocytosis [44•]. These observations on ARF GAP1 and Git1 suggest that some ARF GAPs may be regulated by seven-pass transmembrane receptors to influence selective membrane trafficking pathways (see also update).

ASAP1, the first non-Golgi ARF GAP to be characterized, localizes to focal adhesions and cycles along with other focal adhesion proteins, such as paxillin, into cortical actin ruffles, which are generated in response to growth factors [30•]. Furthermore, overexpression of ASAP1 inhibits cell spreading and ruffling, and this inhibition is dependent upon the GAP activity of ASAP1 [30•]. Several other ARF GAPs have been identified through interactions with focal adhesion proteins, including Pkl and Cat1/2, which interact with paxillin [45•] and Cool/PIX (GEFs for Cdc42) [46•], respectively. The presence of these ARF GAPs in focal adhesions may be linked to the observation that ARF1-GTP stimulates recruitment of paxillin from a juxtanuclear region to focal adhesions [47]. As ARF6 has also been implicated in the cortical actin rearrangements associated with cell spreading and membrane ruffling, this suggests that several ARFs and GAPs at the PM coordinate membrane traffic and actin structures. Interestingly, the yeast GAP Gcs1 regulates the actin cytoskeleton *in vivo*, and *in vitro* it binds to actin directly [48•].

Effectors

Increasing attention is being paid to the roles of ARF in lipid modification. In yeast, the putative aminophospholipid translocase Drs2 plays an important role in ARF-mediated clathrin-coated vesicle formation at the TGN [49•]. Perhaps this is through direct effects on lipid-bilayer composition [49•]. An exciting development over the last year has been the identification of a new class of lipid-modifying enzymes as ARF effectors: the PI(4)P 5-kinases. This finding is particularly interesting in light of studies demonstrating a role for $PI(4,5)P_2$ (PIP_2), and the enzymes responsible for its synthesis, in trafficking steps at the Golgi and at the PM [3]. ARF1, ARF5 and ARF6, which represent all three classes of ARFs, activate mouse PI(4)P 5-kinase α *in vitro* [50•]. At the PM, ARF6 colocalizes with mouse PI(4)P 5-kinase α on the membrane ruffles induced by aluminum fluoride treatment or EGF stimulation [50•]. At the Golgi complex, PI 4-kinase β and an unidentified PI(4)P 5-kinase activity are recruited to Golgi membranes by activated ARF1, leading to an increase in the production of PIP_2 [51•]. In addition, recombinant PI(4)P 5-kinase stimulates PIP_2 production on Golgi membranes in the presence of ARF1 [52•]. A yeast PI 4-kinase β homologue, Pik1, shows genetic interactions with ARF and is required for transport pathways from the Golgi, indicating a conserved role for these lipid kinases in Golgi function [53].

As members of all classes of ARF can stimulate PI(4)P 5-kinase, this lipid-modulating function is likely to be a general consequence of ARF activation. PIP_2 can, in turn, stimulate the activity of some ARF GAPs [54], thus creating a negative-feedback loop to turn off ARF and PIP_2 production. PIP_2 is an important membrane lipid that influences numerous events in the cell, in addition to membrane traffic, including actin dynamics and signalling cascades at the plasma membrane [55]. Hence, a fundamental role of ARF may be to coordinate membrane dynamics and other cellular functions through its interaction with lipid kinases.

Another exciting recent advance is the discovery of a new class of ARF effectors: the GGA proteins (Golgi-localizing, gamma adaptin ear homology domain, ARF-binding proteins). Boman *et al.* [56•] identified GGA1 and GGA2 in a yeast two-hybrid screen using the constitutively activated Q71L mutant of ARF3. These two proteins, along with a third, GGA3, were also identified in the gene data banks by their homology to the ear region of γ-adaptins [57•,58•]. Unlike the known coats that are part of oligomeric complexes, the GGA proteins are monomeric when purified from the cytosol [57•,58•]. Overexpression of any one of the GGA proteins perturbs trafficking of TGN-resident proteins, releases AP-1/clathrin from membranes and, at high levels of overexpression, releases the COPI coat from Golgi membranes [56•–58•]. There are two yeast homologues that have an overlapping function in transport from the TGN to the vacuole/lysosome [57•,58•]. The GGA proteins all have an amino-terminal VHS (Vps27, Hrs and STAM) domain, followed by the GAT (GGA1 and TOM proteins) region, and, at the extreme carboxyl terminus, the γ-adaptin homology domain [56•–58•]. The two yeast proteins have the

same domain organization and a high overall level of sequence similarity, suggesting a conserved function [57•,58•]. The GAT domain binds ARF-GTP directly and is responsible for localization of the GGA proteins to the TGN [57•]. Whether the GGAs form part of a novel coat or regulate the assembly of other coats remains to be established.

Analyses of mutations in class I ARFs have revealed that regions important for coatomer recruitment to membranes do not coincide with those for activation of phosphlipase D (PLD), and hence, COPI coat recruitment and PLD stimulation by ARF can be uncoupled [59,60•]. Although coat recruitment to membranes is an important consequence of Class I ARF activation [4,61], recent work has underscored the importance of other effector functions. Both Golgi and endosome *in vitro* transport assays are blocked by ARF-GTPγS, even in the absence of coat protein complexes in the assays [62•,63•]. Candidates for other effectors are PLD, PIP kinases (see above), arfaptin1 and aftaptin2/POR1 (see [1]). Two other potential effectors, specific for class I ARFs, are the kinesin-like protein MKLP1 [64] and the PDZ-domain-containing PICK1 protein [65]. Arfophilin is another recently identified potential ARF effector, which interacts specifically with activated ARF5, a class II member [66].

Conclusion

Over the past year, the identification of novel regulators and effectors for ARF GTPases has begun to reveal their broad range of functions. An emerging theme is the importance of ARF in generating specific lipid modifications and in coordinating membrane dynamics and actin function. For both the GEFs and the GAPs defining their ARF specificity, localization and regulation will be critical for understanding how ARFs regulate membrane dynamics within the cell.

Update

Recent work has implicated ARF6 and its GEF, ARNO, in desensitization (internalization) of the luteinizing hormone (LH)/choriogonadotropin receptor [68]. The results suggest that LH binding to this seven-pass transmembrane receptor would activate an ARF6 GEF such as ARNO, which in turn would activate ARF6, leading to increased binding of β-arrestin to the receptor [68]. These results provide further evidence of coordinated regulation of ARF with seven-pass transmembrane receptor signalling pathways and, in particular, mirrors the inhibition of receptor internalization observed for Git1 overexpression, a GAP for ARF6 [44•]. This work, in addition to another recent paper demonstrating that ARNO activates ARF6 in chromaffin cells [69], also supports the idea that ARNO can act as a GEF for ARF6 *in vivo*.

Some questions exist as to the role of COPI in ARF GAP1-stimulated GTP hydrolysis. Szafer *et al.* [70] found that COPI does stimulate GTP hydrolysis on truncated, non-myristoylated ARF1 as observed by Goldberg [32,34•], but that when full-length myrARF1 is used as a substrate in the presence of phospholipid micelles, no COPI-stimulation of GAP activity is observed. These new observations do not preclude a role for COPI in modulating the ARF GTPase cycle. Ultimately, however, the physiological roles of ARF GAP1, COPI, and cytoplasmic tails of cargo receptors on the ARF-GTP-hydrolysis step will have to be assessed on membrane surfaces where ARF-GTP resides.

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- •• of outstanding interest

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This study builds on previous ones showing that the carboxy-terminal portion of ARF GAP1 has sequences required for targeting to Golgi membranes. Here, the authors show that these Golgi-targeting sequences are also required for ARF GAP1 association with the KDEL receptor, Erd2. In cells expressing a mutant form of Erd2 that is defective in GAP binding the authors show that ARF1-GFP has a longer dissociation time from Golgi membranes after BFA treatment. This would suggest a reduced level of ARF GAP activity on the Golgi.

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Evidence is provided that Gcs1 and Glo3 have overlapping functions in ER–Golgi transport in yeast. Yeast with loss of both GAPs have a growth defect, accumulate ER, and have defects in ER–Golgi transport. These effects can be rescued by expression of rat ARF GAP1. Gcs1 and Glo3 are essential membrane components of an *in vitro* Golgi–ER retrograde transport assay.

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• Nilsson T: **GTP hydrolysis by arf-1 mediates sorting and concentration of Golgi resident enzymes into functional COP I vesicles.** *EMBO J* 1999, **18**:4935-4948.
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Extending previous studies, these authors show that Golgi-derived COPI-coated vesicles obtained in the presence of GTP contain more anterograde cargo molecules than those obtained in the presence of GTPγS or the constitutively active mutant of ARF1, Q71L.

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• **GIT proteins, a novel family of phosphatidylinositol 3,4,5-triphosphate-stimulated GTPase-activating proteins for ARF6.** *J Biol Chem* 2000, **275**:13901-13906.
These authors demonstrate that Git1 and Git2 stimulate GTP hydrolysis on all classes of ARFs, including ARF6, and are the first ARF GAPs shown to have such activity on ARF6. Unlike ARF GAP1, Git1 and Git2 activity is stimulated by PIP_3.

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Cat1 and Cat2 were identified by two-hybrid interaction with Cool/PIX. Cat1 and 2 become phosphorylated when cells are plated on fibronectin and both can be phosphorylated by src and focal adhesion kinase (FAK).

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• Drubin DG, Prestwich GD, Theibert AB: **GCS1, an Arf guanosine triphosphatase-activating protein in Saccharomyces cerevisiae, is required for normal actin cytoskeletal organization *in vivo* and stimulates actin polymerization *in vitro*.** *Mol Biol Cell* 1999, **10**:581-596.
This study supports a role for ARF GAPs in actin organization in yeast. Deletion of Gcs1, an ARF GAP implicated in ER-Golgi trafficking in yeast (see [38]), results in altered actin structures, increased sensitivity to latrunculin B, and synthetic lethality with deletion of *SLA2*, a gene implicated in actin stabilization. In addition to a GAP domain, Gcs1 has PH and ERM (ezrin–radixin–moesin) domains. Gcs1 is also shown to bind to actin filaments and to stimulate actin polymerization *in vitro*.

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These authors identified *drs2Δ* as a mutant that is synthetically lethal with an *arf1Δ* mutant in yeast. Yeast cells lacking Drs2p (*drs2Δ*) have defects in transport from the TGN to the endosome and from the endosome to the vacuole/lysosome. Strikingly, when fractions normally enriched in clathrin-coated vesicles were prepared from *drs2Δ* cells, empty clathrin baskets lacking a lipid bilayer were found, indicating that Drs2p plays an essential role *in vivo* in formation of clathrin-coated vesicles from the TGN.

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• Watanabe H, Kawamoto K, Nakayama K, Morris AJ, Frohman MA, Kanaho Y: **Phosphatidylinositol 4-phosphate 5-kinase alpha is a downstream effector of the small G protein ARF6 in membrane ruffle formation.** *Cell* 1999, **99**:521-532.
Setting out to purify activators of PI4P 5-kinase present in brain cytosol, Honda *et al*. identified ARF proteins. All ARFs when GTP-bound *in vitro* could activate PI4P 5-kinase, generating $PIP(4,5)_2$. In cells, however, there is a correlation between localization of PIP 5-kinase and ARF6, suggesting that PIP 5-kinase is a downstream effector for ARF6 *in vivo*. This is consistent with other studies that demonstrate an influence of ARF6 on the actin cytoskeleton.

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These authors show that ARF1 recruits PI 4-kinase-β and an unidentified PIP 5-kinase to the Golgi complex, leading to dramatic stimulation of PIP_2 at the Golgi upon ARF activation. Overexpression of a dominant-negative PI 4-kinase-β mutant affected Golgi morphology in mammalian cells, leading to a more disorganized, punctate immunofluorescence staining pattern for two Golgi-localized proteins.

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• **Type 1 phosphatidylinositol 4-phosphate 5-kinase directly interacts with ADP-ribosylation factor 1 and is responsible for phosphatidylinositol 4,5 biphosphate synthesis at the Golgi compartment.** *J Biol Chem* 2000, in press.
Jones *et al*. show that Golgi-membrane preparations have no associated activity that synthesizes PIP_2, but PIP_2 production can be stimulated by adding ARF1, GTPγS and recombinant PIP 5-kinase. ARF pretreatment of Golgi membranes leads to an increase in PIP_2 through phosphorylation of PI(4)P by PIP 5-kinase.

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This paper reports on a set of new ARF1-interacting proteins, the GGAs (Golgi-localizing, Gamma-adaptin ear homology domain, ARF-binding proteins), which reversibly associate with the TGN in a BFA-sensitive manner. These authors identified these interesting proteins in a yeast two-hybrid screen and demonstrate that the GGAs directly bind to GTP-bound ARF1 and 3, and not to GDP-bound forms. The GGAs are expressed in all tissues.

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See annotation [58•].

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• **A family of proteins with γ-adaptin and VHS domains that facilitate trafficking between the trans-Golgi network and the Vacuole/Lysosome.** *J Cell Biol* 2000, **149**:67-80.
These two papers [57•,58•] investigate the gamma-adaptin like proteins, the GGAs. Both studies show that the GGA proteins associate with the TGN as monomers. Dell'Angelica *et al*. [57•] defined an amino-terminal region of the molecule, the GAT domain, that is required for Golgi targeting and ARF-GTP association. Overexpression of this GAT region caused dissociation of the ARF-dependent coat proteins (AP1, AP3, AP4 and COPI) from Golgi and endosomal membranes. Hirst *et al*. [58•] also showed that the GGAs are not components of AP1-clathrin-coated vesicles and that in yeast the loss of the two GGAs causes aberrant vacuolar morphology. Both groups showed missorting of the vacuolar hydrolase CPY in *gga1Δ gga2Δ* double mutants.

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Point mutations in switch I and II regions of ARF1 were analyzed both in yeast two-hybrid interaction screens and in PLD and COPI-binding assays *in vitro*. The effects of the different mutants argues for uncoupling of PLD and COPI binding activities. Furthermore, the ability of ARF1 mutants to bind differentially to different effectors (POR1, MKLP and GGA1) suggests distinct sites of interaction.

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• **Studies on the inhibition of endosome fusion by GTPgammaS-bound ARF.** *J Cell Sci* 1999, **112**:3477-3485.
See annotation [63•].

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